UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
WASHINGTON, DC 20460

OFFICE OF CHEMICAL
SAFETY AND POLLUTION
PREVENTION

January 21, 2016

MEMORANDUM

Subject: Protocol Review for 1706PA4 (Standard Operation Procedure for Measuring Antimicrobial Efficacy in Potable Water System); DB Barcode: D430459.

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Applicant: Nalco Company
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I. BACKGROUND

Nalco, An Ecolab Company is resubmitting a testing protocol for Measuring Antimicrobial Efficacy in Institution Potable Water Systems following rejection of the initial for not meeting 6 log_{10} required for bacteria. The registrant is modifying ASTM E-645 "Evaluation of Microbes Used in Cooling Water Systems" to access efficacy of secondary potable water treatment methods against bacteria mainly *Legionella pneumophila* (ATCC 33152) and *Pseudomonas aeruginosa* (ATCC 15442). Protocol was developed by Ecolab Microbiological Services.

This data package is identified as D430459 contained a letter from the applicant to EPA (dated October 7, 2015), one protocol (MRID 497418-01).

II. BRIEF DESCRIPTION OF THE PROTOCOL

1. MRID 497418-01 "Standard Operating Procedure for Measuring Antimicrobial Efficacy in Potable Water Systems" by Ecolab Microbiological Services.

The purpose of this test method is to evaluate the efficacy of secondary potable water treatment methods against bacteria.

Method References:

1. AOAC Method 960.09; Germicidal & Detergent Sanitizing Action of Disinfectants, 2013
2. ASTM E645-13; Standard Practice for Evaluation of Microbicides Used in Cooling Water Systems
3. HACK DPD method - equivalent to the US EPA Standard Method 4500-ClO_2P.

Test System (Microorganism):

1. *Legionella pneumophila* ATCC 33152 (Strain: Philadelphia-1)
2. *Pseudomonas aeruginosa* ATCC 15442
3. Other bacteria may be tested at the discretion of the investigator

Procedure:

1. Dispense 99 mL of each test solution into a sterile 250 mL Erlenmeyer flask. Prepare duplicate flasks for each test solution to be tested and keep in water bath at 20 ± 2°C.
2. Also prepare duplicate flasks with 99 mL of the sterile potable water for enumeration of initial numbers control for each test system and treat in the same manner as the test flasks.
3. At time 0, swirl the flask and add 1.0 mL of the test system to the 99 mL of the test solution midway between the side and the center of the flask with the tip slightly immersed in the test substance.
4. Avoid touching the sides of the flask with the pipet.
5. After exposure times of 5 min, 15 min, and 30 min, remove 1.0 mL of test substance/test system mixture and add to 9.0 mL of neutralizer. This is the 10^{-1} dilution. Other exposure times may be used.

After each exposure time, remove a second 1 ml aliquot and test for free residual oxidant using the HACK DPD titration method for the biocide being tested

6. Make serial 10 fold dilutions and plate in duplicate onto appropriate media. Suggested dilutions to plate for number controls are 10^{-5}, 10^{-6} and 10^{-7}. Dilutions for test samples will depend upon concentrations and exposure times. It is recommended to plate all dilutions of test sample at 0.1 ml for 0-10^{-3} and 1 ml for 10^{-4} – 10^{-6}. For control samples, 0.1 mf of the solution can be plated for 10^{-5} and 10^{-6} dilutions.
7. All plates should be incubated for 5 ± 1 days at 35 ± 2°C. [This incubation time and temperature is for Legionella and will vary based on the test organism]

Success Criteria:
1. For organisms of public health concern at a public health site, efficacy will be gauged to a 6-log reduction in a period not to exceed 24 hours
2. For *Legionella pneumophila* reduction at a public health site the efficacy will gauged to a 5-log reduction in a period not to exceed 24 hours
3. This protocol may also be used for evaluating the efficacy of an antimicrobial agent in a non-public health site application

III. CONCLUSION AND COMMENTS

1. The submitted protocol (MRID 497418-01) **is adequate** for evaluating the efficacy of secondary potable water treatment methods against bacteria (*Legionella pneumophila* and others). **However, treatment contact time period is not to exceed 30 minutes.**